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(54) Title: <b>DELTA 6 FATTY ACID DESATURASE</b>			
(57) Abstract <p>Novel human DNA sequences that encode the gene CYB5RP, a delta 6 fatty acid desaturase, are provided. Provided are genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. Also provided is CYB5RP protein encoded by the novel DNA sequences. Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are CYB5RP methods that identify activators and inhibitors of CYB5RP protein.</p>			

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**TITLE OF THE INVENTION**  
**DELTA 6 FATTY ACID DESATURASE**

**CROSS-REFERENCE TO RELATED APPLICATIONS**

5           Not applicable.

**STATEMENT REGARDING FEDERALLY-SPONSORED R&D**

Not applicable.

10          **REFERENCE TO MICROFICHE APPENDIX**

Not applicable.

**FIELD OF THE INVENTION**

15          The present invention is directed to novel human DNA sequences encoding a delta 6 fatty acid desaturase, an enzyme involved in the synthesis of essential fatty acids.

**BACKGROUND OF THE INVENTION**

20          Essential fatty acids (EFAs) are polyunsaturated fatty acids that cannot be manufactured by mammals, yet are required for a number of important biochemical processes, and thus must be supplied in the diet. The most important dietary EFAs are linoleic acid and alpha-linolenic acid (ALA). These two EFAs undergo a number of biosynthetic reactions that convert them into various other EFAs. Figure 1 depicts the biosynthetic reactions involving the two groups of EFAs, the n-6 EFAs (linoleic acid derivatives) and the n-3 EFAs (ALA derivatives). EFAs are formed from linoleic acid and ALA by a series of alternating reactions involving the removal of two hydrogens coupled with the insertion of an additional double bond (desaturation) and the lengthening of the fatty acid chain by the addition of two carbons (chain elongation). The enzymes catalyzing the desaturations and elongations are thought to 25 be the same for both groups of EFAs.

30          Among the more important unsaturated fatty acids are the delta 6 unsaturated fatty acids, which are involved in the maintenance of membrane structure and function, the regulation of cholesterol synthesis and transport, and the prevention

of water loss from the skin. Delta 6 unsaturated fatty acids also serve as precursors of the eicosanoids, including the prostaglandins and leukotrienes (Horrobin, 1992, *Prog. Lipid Res.* 31:163-194). The double bond at the 6 position of delta 6 unsaturated fatty acids is introduced by a class of enzymes known as delta 6 desaturases.

- 5 Deficiencies in linoleic acid and ALA derivatives have been associated with skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction. For example, a deficiency in gamma-linolenic acid (GLA), which is produced from linoleic acid by the action of the enzyme delta 6 desaturase, can arise from the
- 10 decreased activity of this enzyme that occurs in aging, stress, diabetes, eczema, and some infections, or from increased catabolism of GLA due to oxidation or rapid cell division, as occurs in inflammation or cancer. Clinical trials have demonstrated that dietary GLA supplementation can be effective in treating a number of conditions that are associated with GLA deficiency, *e.g.*, atopic eczema, mastalgia, diabetic neuropathy, viral infections, and some forms of cancer (Horrobin, 1990, *Rev. Contemp. Pharmacother.* 1:1-45).
- 15

Delta 6 desaturase is an example of a fatty acid desaturase. Fatty acid desaturases are enzymes that introduce a double bond into the carbon chain of fatty acids. They play vital roles in the biosynthesis of polyunsaturated fatty acids,

20 including the essential fatty acids. Fatty acid desaturases are present in soluble and membrane-associated forms and require electron donors (for example, cytochrome b5) for their functioning.

Delta 6 desaturases catalyze the rate-limiting steps in the biosyntheses of the linoleic and ALA group EFAs shown in Figure 1. End products of the linoleic acid pathway include the eicosanoids (prostaglandins and leukotrienes). The end product of the ALA pathway is docosahexaenoic acid (DHA), an important component of membranes in the vertebrate retina. DHA is highly specific for retina and represents more than 50% of the fatty acids in the rod outer segment (ROS). It appears that DHA is important in maintaining the normal structure and function of the retina (Anderson et al., 1992, *Neurobiology of Essential Fatty Acids*, Bazan et al., eds., Plenum Press, New York, pages 285-294). Increased dietary consumption of DHA and its precursor, eicosapentaenoic acid, from seal meat and fish has been

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linked to an increased incidence of macular degeneration in Greenland Eskimos (Rosenberg, 1987, *Arct. Med. Res.* 46:64-70).

Certain delta 6 desaturases have been cloned from plants. For example, a delta 6 desaturase has been cloned from borage (Sayanova et al., 1997, 5 *Proc. Natl. Acad. Sci. USA* 94:4211-4216). This delta 6 desaturase is unusual in that its cytochrome b5 electron donor is present as an N-terminal extension of the enzyme rather than being synthesized as a separate protein. The borage delta 6 desaturase has been shown to be functional, in that transfer of the cloned gene encoding it to tobacco results in the synthesis of high levels of GLA and octadecatetraenoic acid (OTA) in 10 the transgenic tobacco leaves. GLA and OTA are the products of delta 6 desaturase activity on linoleic acid and ALA, respectively.

Based on its hydropathy profile, the borage delta 6 desaturase appears to be a membrane-bound protein. Examination of the amino acid sequence of the borage enzyme, as well as the amino acid sequences of membrane-bound desaturases 15 from a wide variety of organisms, has revealed three regions of conserved short motifs containing histidine residues (HX(3 or 4)H, HX(2 or 3)HH, and HX(2 or 3)HH) having a conserved spacing from each other (Shanklin et al., *Biochemistry*, 1994, 33:12787-12794).

A DNA sequence has been isolated from sunflower embryos that, 20 judging from its sequence, appears to encode a delta 6 desaturase having a cytochrome b5-like moiety fused to its N-terminus (Sperling et al., 1995, *Eur. J. Biochem.* 232:798-805).

#### SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences that 25 encode a delta 6 fatty acid desaturase, cytochrome b5-related protein (CYB5RP). The present invention includes genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. The genomic CYB5RP DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The cDNA 30 encoding CYB5RP protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2. Also provided is CYB5RP protein encoded by the novel DNA sequences. The CYB5RP protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3.

Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are methods of producing delta 6 unsaturated fatty acids using DNA encoding CYB5RP or using CYB5RP protein.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the enzymatic conversions involved in the linoleic acid (n-3) and alpha-linolenic acid (n-6) pathways of essential fatty acid synthesis.

Figure 2A-G shows the genomic DNA sequence of the CYB5RP gene (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon at position 544 in exon 1 and the stop TGA codon at position 18,103 in exon 10 12 are shown in bold. The putative polyadenylation signal ATTAAA located approximately 20 base pairs upstream of the polyA tail is shown in bold italics (position 18,373 in exon 12). DNA sequence upstream of exon 1 represents a putative promoter region of the CYB5RP gene., as indicated by the presence of the 15 TATA box at position 353 (underlined bold)..

Figure 3A-C shows the cDNA sequence (SEQ.ID.NO.:2) and the amino acid sequence (SEQ.ID.NO.:3) of CYB5RP. The region encompassing amino acids 1-102 represents the cytochrome b5 domain. The region encompassing amino acids 182-186 represents HIS BOX 1. The region encompassing amino acids 219-223 20 represents HIS BOX 2. The region encompassing amino acids 383-387 represents HIS BOX 3.

Figure 4 shows a portion of the cDNA sequence (SEQ.ID.NO.:4) and a portion of the amino acid sequence (SEQ.ID.NO.:5) of mouse CYB5RP.

Figure 5A shows a Kyte-Doolittle hydropathy plot of CYB5RP. 25 Figure 5B shows the proposed membrane topology of CYB5RP based on its hydropathy plot. This membrane topology is similar to that proposed for other membrane-bound fatty acid desaturases (Shanklin et al., Biochemistry, 1994, 33:12787-12794). The amino acids shown in Figure 5B are portions of (SEQ.ID.NO.:3).

Figure 6 shows the output of the Profilescan program from the 30 Wisconsin GCG package. The upper amino acid sequence is from CYB5RP (positions 31-78 of SEQ. ID. NO.3). The lower amino acid sequence is positions 1-48 of the cytochrome b5 profile (SEQ. ID. NO.:6.). The output shows that CYB5RP

contains a profile typical for the heme-binding domain of the cytochrome b5 protein family. Importantly, the region of identity includes the invariant HPGG motif, where histidine represents a heme axial ligand for iron.

Figure 7A and B show the results of BlastP searches of the GenBank database using the full-length CYB5RP amino acid sequence as the query. Figure 7A shows the hit with highest homology, a hypothetical protein from sunflower. The sunflower protein and CYB5RP share three His boxes (boxed) in which the spacing between the His boxes is conserved. Also boxed is the HPGG motif typical for the heme-binding domain of the cytochrome b5 protein family. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.3. The lower amino acid sequences shown are portions of the amino acid sequence of the hypothetical protein from sunflower (Sperling et al., 1995, Eur. J. Biochem. 232:798-805). The sequence shown as positions 348-432 is SEQ. ID. NO.:7. The sequence shown as positions 22-74 is SEQ. ID. NO.:8. The sequence shown as positions 152-227 is SEQ. ID. NO.:9. Figure 7B shows the hit with the second highest homology, a delta 6 desaturase from *Borago officinalis* (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216). The *Borago* protein and CYB5RP also share three His boxes with conserved spacing, as well as the HPGG motif. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.3. The lower amino acid sequences shown are portions of the amino acid sequence of the *Borago* delta 6 desaturase. The sequence shown as positions 338-424 is SEQ. ID. NO.:10. The sequence shown as positions 12-64 is SEQ. ID. NO.:11. The sequence shown as positions 153-220 is SEQ. ID. NO.:12.

Figure 8 shows additional results of BlastP searches of the GenBank database using the CYB5RP protein as the query. Figure 8 shows the amino acid alignment between the CYB5RP protein and a delta 6 desaturase from *Synechocystis* sp. (strain pcc 6803) performed by the BlastP program. The *Synechocystis* delta 6 desaturase and CYB5RP share three His boxes, two of which are shown in Figure 8 (boxed). In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The CYB5RP

sequence shown is a portion of SEQ. ID. NO.3. The *Synechocystis* sequence shown is SEQ. ID. NO:13.

Figure 9A shows the expression pattern of the CYB5RP gene in 9 human tissues, as determined by RT-PCR amplification with 21 cycles. Expression is 5 detected in human retina, kidney, pancreas, placenta, and brain. Figure 9B shows the results of the analogous experiments performed with 25 cycles of amplification. Expression of the CYB5RP gene is seen in all the human tissues studied.

#### DETAILED DESCRIPTION OF THE INVENTION

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For the purposes of this invention:

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

The present invention relates to the identification and cloning of cytochrome b5-related protein (CYB5RP), a gene which encodes a human delta 6 fatty acid desaturase. The gene is present on PAC clones 759J12, 756B3, 519O13, and 466A11 from an area of human chromosome 11q12 that has been shown to contain a gene related to Best's macular dystrophy (Cooper *et al.*, 1997, Genomics 41:185-192; Stöhr *et al.*, 1997, Genome Res. 8:48-56; Graff *et al.*, 1997, Hum. Genet. 101: 263-279). This linkage between the chromosomal location of the CYB5RP gene and the location of the gene related to Best's macular dystrophy can be used diagnostically by identifying restriction fragment length polymorphisms (RFLPs) in the vicinity of the CYB5RP gene, *e.g.*, in SEQ.ID.NO.:1. Such RFLPs will be associated with the Best's macular dystrophy gene and thus can be used to identify individuals carrying disease-causing forms of the Best's macular dystrophy gene.

CYB5RP was identified as an EST hit in sequence scanning data from PAC clones from human chromosome 11q12. In addition, a full length cDNA of CYB5RP was recovered from a human retina cDNA library. The genomic region of CYB5RP has been sequenced and the exon/intron organization of CYB5RP has been determined. The CYB5RP gene has 12 exons. The promoter region of CYB5RP was identified upstream of the 5' UTR by detecting consensus elements required for eukaryotic transcription. The expression pattern of CYB5RP was determined by RT-PCR analysis in 9 human tissues. The CYB5RP gene is expressed predominantly in human retina, kidney, pancreas, and placenta; lower levels of expression are also detected in brain, heart, lung, liver, and skeletal muscle. Bioinformatic analysis revealed significant homology to a group of plant and bacterial fatty acid desaturases. All of the typical amino acid motifs present in these fatty acid desaturases are also present in CYB5RP. Kyte-Doolittle algorithm analysis predicts a transmembrane organization typical of fatty acid desaturases for CYB5RP (see Figure 5). CYB5RP is

unusual in that it contains a cytochrome b5 region in its N terminus. While many fatty acid desaturases utilize cytochrome b5 as an electron donor, most have not incorporated this cytochrome as part of their polypeptide chain.

That CYB5RP is a fatty acid desaturase is shown by the following evidence:

- (1) CYB5RP possesses significant homology to a group of plant and microbial fatty acid desaturases;
- (2) Like other fatty acid desaturases, CYB5RP has three conserved histidine boxes, with correct spacing between the boxes; and
- (3) The predicted membrane topology of CYB5RP is similar to that of known fatty acid desaturases.

That CYB5RP is a delta 6 fatty acid desaturase is shown by the following evidence:

- (1) CYB5RP contains a cytochrome b5-like moiety fused to its N-terminus. The only two fatty acid desaturases that contain cytochrome b5-like moiety fused to their N-termini are known or suspected to be delta 6 desaturases.
- (2) The only two plant desaturases that are known or suspected to introduce a double bond in the 6 position have an atypical His box 3 (QI/LEHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.
- (3) The only bacterial desaturase that is known to introduce a double bond in the 6 position has an atypical His box 3 (QVTHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.

CYB5RP is a target for the development of drugs for the treatment of disorders of lipid metabolism and for the treatment of conditions that require the modulation of the biosynthesis of prostaglandins and leukotrienes (asthma, pain, etc.). CYB5RP is also a target for the development of drugs for use in treating skin diseases, diabetic complications, reproductive disorders, including breast pain and premenstrual syndrome, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infections, and various forms of retinal degeneration, including age-related macular degeneration.

CYB5RP is homologous to a delta 6 desaturase from *Borago officinalis* (see Figure 7B). Both CYB5RP and this *Borago* delta 6 desaturase, unlike desaturases from higher plants, are unusual in containing a cytochrome b5-like

domain fused to their N-termini (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216; hereinafter "Sayanova"). The *Borago* desaturase has been expressed in transgenic tobacco, resulting in high levels of delta 6 desaturated fatty acids in the transgenic tobacco leaves, including high levels of  $\gamma$ -linolenic acid (GLA) (Sayanova).

- 5 Given the medical importance of GLA, Sayanova proposed that transgenic plants, expressing the *Borago* delta 6 desaturase, would be valuable as sources of GLA. Similarly, CYB5RP, expressed in transgenic plants, is expected to provide a valuable source of GLA.

The present invention provides DNA encoding CYB5RP that is substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding CYB5RP. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 2 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 12 exons. These exons collectively have an open reading frame that encodes a protein of 445 amino acids. When an alternatively spliced exon 8 is used, a CYB5RP protein of 433 amino acids, lacking amino acids 317-328, is produced. Thus, the present invention includes two cDNA molecules, encoding two forms of CYB5RP protein, that are substantially free from other nucleic acids. The first cDNA is shown in Figure 3 and has the nucleotide sequence SEQ.ID.NO.:2. The second cDNA is identical to the first, except that it does not contain the nucleotides at positions 1,019-1,054.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding region of SEQ.ID.NO.:2. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2. The present invention also includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2, except that the nucleotides at positions 1,019-1,054 are missing. Also included in the present invention are recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 and recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 with the exception that positions 1,019-1,054 are missing.

The novel DNA sequences of the present invention encoding CYB5RP, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which CYB5RP is not naturally linked, to form "recombinant DNA molecules" encoding CYB5RP. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NOs.:1 or 2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows:

15 Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in a prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 hr in a 20

20 solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 25 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor 30 Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the

construction of synthetic DNA that encodes the CYB5RP protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:2, but still encodes the same CYB5RP protein shown as SEQ.ID.NO.:3. Such synthetic DNAs are intended to be within the scope of the present invention. Also with the scope of the present invention are synthetic DNAs that encode a CYB5RP protein lacking amino acids 317-328 of SEQ.ID.NO.:3.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding CYB5RP protein. Such recombinant host cells can be cultured under suitable conditions to produce CYB5RP protein. An expression vector containing DNA encoding CYB5RP protein can be used for expression of CYB5RP protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, plant cells such as tobacco, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of CYB5RP protein and which are commercially available, include but are not limited to, L cells L-M(TK<sup>-</sup>) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

A variety of mammalian expression vectors can be used to express recombinant CYB5RP in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNA1 and pcDNA1amp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, CYB5RP can be purified by conventional techniques to a level that is substantially free from other proteins. A description of vectors that can be used to express CYB5RP can be found in, e.g., Goeddel, ed., 1990, Meth. Enzymol. vol. 185 or Perbal, 1988, A Practical Guide to Molecular Cloning, John Wiley and Sons, Inc.

The present invention includes CYB5RP protein substantially free from other proteins. The amino acid sequence of the full-length CYB5RP protein is shown in Figure 3 as SEQ.ID.NO.:3. Thus, the present invention includes CYB5RP protein substantially free from other proteins having the amino acid sequence

- 5 SEQ.ID.NO.:3. Also included in the present invention is a CYB5RP protein that is produced from an alternatively spliced CYB5RP mRNA where the protein has the amino acid sequence of SEQ.ID.NO.:3 with the exception that amino acids 317-328 are missing.

As with many proteins, it is possible to modify many of the amino acids of CYB5RP and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified CYB5RP proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as CYB5RP. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the His boxes of CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the sunflower protein depicted in Figure 1 of Sperling *et al.*, 1995, Eur. J. Biochem. 232:798-805 when these two proteins are aligned by BLASTP analysis. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the

CCCTCTACCCCTGTCCCATCAGGC (SEQ.ID.NO.:15)

One of skill in the art would recognize that many other primer pairs based upon SEQ.ID.NO.:2 would also be suitable.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl<sub>2</sub>, 200 µM for each dNTP, 50 mM KCl, 0.2 µM for each primer, 10 ng of DNA template, 0.05 units/µl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding CYB5RP can be isolated would be Human Retina 5'-stretch cDNA library in lambda gt10 or lambda gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of either 445 amino acids (SEQ.ID.NO.:3) or an open reading frame of 433 amino acids (SEQ.ID.NO.:3 lacking the amino acids at positions 317-328) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). CYB5RP protein can then be produced by transferring an expression vector encoding CYB5RP or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. CYB5RP protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding CYB5RP can be isolated from a cDNA library using as a probe oligonucleotides specific for CYB5RP and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described

in, e.g., Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for CYB5RP and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of CYB5RP shown in SEQ.ID.NO.:2 and can be synthesized by methods well-known in the art.

Genomic clones containing the CYB5RP gene can be obtained from commercially available human PAC or BAC libraries available from Research Genetics, Huntsville, AL. PAC clones containing the CYB5RP gene (e.g., PAC clones 759J12, 756B3, 519O13, and 466A11) are commercially available from Research Genetics, Huntsville, AL (Catalog number for individual PAC clones is RPCI.C). Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the CYB5RP can be isolated, using probes based upon the CYB5RP sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).

The present invention also provides oligonucleotide probes, based upon SEQ.ID.NO.:2 that can be used to determine the level of CYB5RP RNA in a sample. In particular, the present invention includes DNA oligonucleotides comprising at least 18 contiguous nucleotides of SEQ.ID.NO.:2. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide probes can be packaged in kits.

In addition to the utilities described above, the present invention makes possible the recombinant expression of the CYB5RP protein in various cell types. In particular, it is advantageous to recombinantly express CYB5RP in plant cells. Such expression in plant cells provides a method for the production of high levels of valuable EFAs such as GLA and OTA in the recombinant plant cells. An example of such recombinant expression of a delta 6 fatty acid desaturase, in that case from borage, is described in Sayanova *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94:4211-4216 (Sayanova). The recombinant expression of the borage delta 6 desaturase led to the production of high levels of GLA and OTA in the leaves of the tobacco plants in which it was expressed. The procedures described in Sayanova can be easily adapted to express CYB5RP in tobacco, thus providing an additional, useful way to produce

large amounts of valuable EFAs. Known methods of recombinantly expressing genes in other plant species beside tobacco can be used to express CYB5RP in those other species.

The present invention also makes possible the development of assays 5 which measure the biological activity of the CYB5RP protein. Such assays using recombinantly expressed CYB5RP protein are especially of interest.

Assays for CYB5RP protein activity can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of CYB5RP protein. Such identified compounds can 10 serve as "leads" for the development of pharmaceuticals that can be used to modulate the activity of CYB5RP in patients suffering from conditions where that activity is abnormal, e.g., skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction such as macular degeneration.

Such assays may comprise:

- (a) recombinantly expressing CYB5RP protein in a host cell;
  - (b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;
- 20 where a change in the biological activity of the recombinantly expressed CYB5RP protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CYB5RP protein.

In particular embodiments, the biological activity of the recombinantly 25 expressed CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid or alpha-linoleic acid.

In some embodiments, it may be advantageous to insert additional steps between steps (a) and (b). Such additional steps might include lysing the host cell and fractionating its contents in order to partially purify the recombinantly 30 expressed CYB5RP, thus facilitating exposure of the recombinantly expressed CYB5RP to the substance as well as to any substrate used in the assay.

The present invention includes activators and inhibitors identified by the methods described herein as well as pharmaceutical compositions comprising

such activators and inhibitors. The activators and inhibitors are generally combined with pharmaceutically acceptable carriers before use to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the activator or inhibitor.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where CYB5RP activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection.

Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route

of administration; the renal, hepatic and cardiovascular function of the patient; and the particular composition thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision 5 in achieving concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

The present invention also includes antibodies to the CYB5RP protein. 10 Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire CYB5RP protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, e.g., serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. 15 See, e.g., Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, CYB5RP protein or an 20 antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, e.g., rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of 25 antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, CYB5RP protein or an 25 antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of 30 monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce CYB5RP polypeptides into the cells of target organs, e.g., the pigmented epithelium of the retina or other parts of the

retina. Nucleotides encoding CYB5RP polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding CYB5RP polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with CYB5RP polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate CYB5RP activity.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

**WHAT IS CLAIMED:**

1. A recombinant DNA molecule encoding a polypeptide having the amino acid sequence of SEQ.ID.NO.:3.

5

2. A recombinant DNA molecule comprising a nucleotide sequence selected from the group consisting of:

SEQ.ID.NO.:1;

SEQ.ID.NO.:2;

10 SEQ.ID.NO.:2 lacking positions 1,019-1,054;

positions 71-1,405 of SEQ.ID.NO.:2; and

positions 71-1,405 of SEQ.ID.NO.:2 lacking positions 1,019-1,054.

15 3. A DNA molecule that hybridizes under stringent conditions to the DNA molecule of claim 2.

4. An expression vector comprising the DNA of  
claim 1.

20 5. A recombinant host cell comprising the DNA of claim 1.

6. A CYB5RP protein, substantially free from other proteins, having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:3 and SEQ.ID.NO.:3 lacking positions 317-328.

25

7. The CYB5RP protein of claim 6 containing a single amino acid substitution.

30 8. The CYB5RP protein of claim 7 where the substitution is a conservative substitution.

9. The CYB5RP protein of claim 6 containing amino acid substitutions where the substitutions do not occur in positions where the amino acid

present in CYB5RP at those positions is also present in the corresponding position in the delta 6 desaturase from sunflower when CYB5RP and the delta 6 desaturase from sunflower are aligned by BLASTP analysis or where the substitutions do not occur in positions where the amino acid present in CYB5RP at those positions is also present  
5 in the corresponding position in the delta 6 desaturase from *Synechocystis* when CYB5RP and the delta 6 desaturase from *Synechocystis* are aligned by BLASTP analysis or where the substitutions do not occur in positions where the amino acid present in CYB5RP at those positions is also present in the corresponding position in the delta 6 desaturase from borage when CYB5RP and the delta 6 desaturase from  
10 borage are aligned by BLASTP analysis.

10. An antibody that binds specifically to the CYB5RP protein of claim 6.
15. 11. A DNA or RNA oligonucleotide probe comprising at least 18 contiguous nucleotides of at least one of the sequences of claim 2.
20. 12. A method for determining whether a substance is an activator or an inhibitor of CYB5RP protein comprising:
  - (a) recombinantly expressing the CYB5RP protein of claim 6 in a host cell;
  - (b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;

25 where a change in the biological activity of the recombinantly expressed CYB5RP protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CYB5RP protein.

- 30. 13. The method of claim 12 where the biological activity of CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid.

14. A pharmaceutical composition comprising an activator or an inhibitor of CYB5RP.

15. A method of treating macular degeneration comprising  
5 administering to a patient an effective amount of the pharmaceutical composition of  
claim 14.

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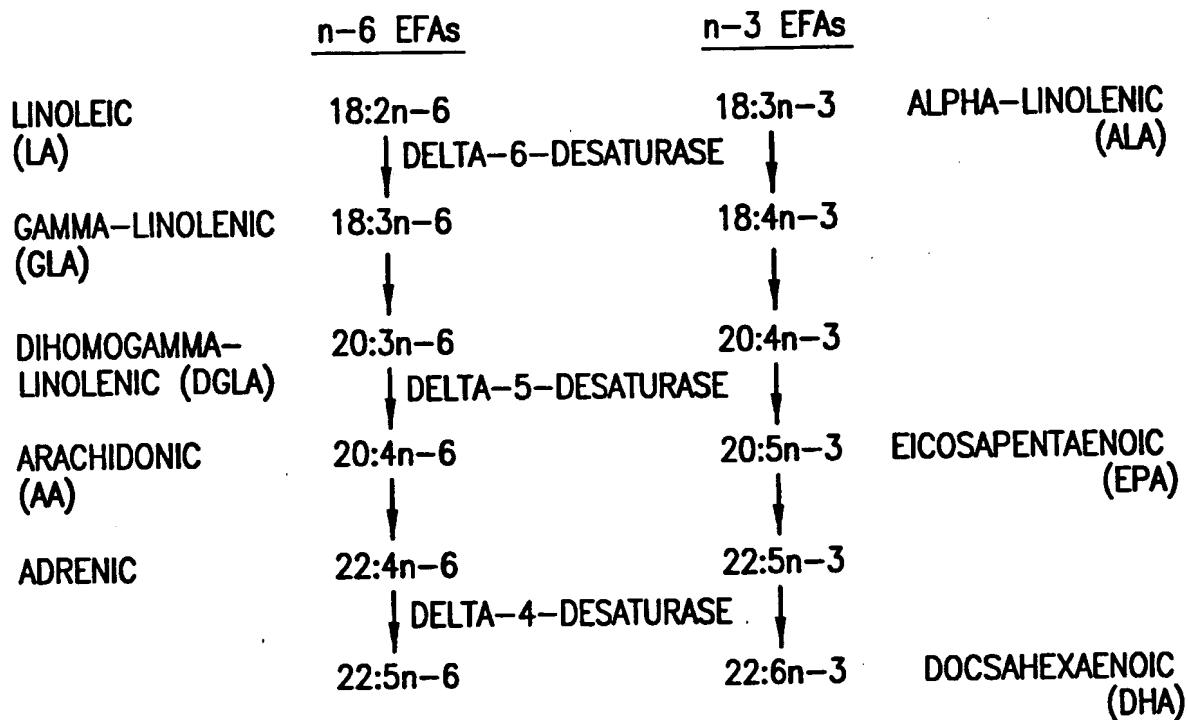


FIG.1

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1 gctcacagac cgggactccg cctccgggtc ccgaggcggt ggcgaggcg  
 51 tgcgggacgc ccaacaggtg cgtttgtgt ccccagggcc cgcgcctccgg  
 101 gtggagtcaa gagcctggaa gccgcagcc cggaaaagg gggcgggacg  
 151 gtgccccggg gcagggctgg gtggcggccg ctgtcctccc gggagggg  
 201 ggccgcctcg acgcccgcct ccctggccgc caatggagac cgaggcccc  
 251 cgcctggatt ggagcggacg cgggggtcag ccagccttgg gggccgggg  
 301 ctggccgggg gcgggggggc aggcgaggcg aggccggcgc cgtccgc  
 351 gtataaaggc ggggagttcc ctgcgccgcg agccgggagg cgcacgc  
 401 ctcgtacggc gggcgccgc gcagggccgg gccggagcag cggcgccgg  
 451 cggaggccgc gcccgggagc gctTTCGCT TCCCTCGGGG TCTTGCTCGG  
 501 ACCTCGGCCA CCGCCTGGGA TCCCCAGGAC TCGTGCCTGC AGCATGGCG  
 551 GCGTCGGGGA GCCGGGACCG CGGGAGGGAC CCGCGCAGCC GGGGGCGCCG  
 601 CTGCCCACCT TCTGCTGGGA GCAGATCCGC GCGCACGACC AGCCC  
 651 CAAGTGGCTG GTCATCGAGC GCCGGTCTA CGACATCAGC CGCTGGGCAC  
 701 AGCGGCACCC AGGGGGCAGC CGCCTCATCG GCCACCACGG CGCTGAGGAC  
 751 GCCACGgtaa ggaagccata aggaagccac ccaccggcgg gtggagcc  
 801 gagctcggtc gtggcgtga tgtccgcgtc cacctgtggg gccttagcat  
 851 cctccctccc ctcgctgacc tttgacctcc acgcccggac ccagagttgg  
 901 ggtggactag ccagggccag atgtggggta gggagggcag ttccctgc  
 951 ggaggaccccg cagctgtcca cggacggat ctgcggggga ggaggggg  
 1001 tcagaggtgg gtgtgtcatg ctgcagagcc tgcctgggt gaggggctgc  
 1051 cctgttgctc ccaggtccct gtttcatgtc tgggtccccca tgctgggtgc  
 1101 ttgctgagtg cttagggtag ggcaggccag gttcccccagg gcccggtaag  
 1151 gacatgccccat tagaggctgg gggctggggcc ggcctgaggt ctgtggctt  
 1201 cccaagagct tctgtaaagg gctcagggac agtactcac ctctccgggc  
 1251 tagcagctgc acgtggggagg gcttgcgcag ccaggctggg tggcctctc  
 1301 ctggaagcac agtcacccca ggaacaggct ggcctgggg gaccccaact  
 1351 tcccaatccc agccctgtc tagacaggca gggatgttagc ctggccccc  
 1401 ggtactgtct ggctggagtc cagtgggtga gcaggccgac cagcccttt  
 1451 tccttagtta cccacactgca taataggggt tggggccac atgcctgtc  
 1501 cttgaccctc caaatcttca gttggggcac actgggtatc aggaaggct  
 1551 tcaagaccccg aggacatgaa tcctgaatgc tggcttttgg ggcagc  
 1601 gaggttctgt ccagtcccg gactgtggc tgcctcttgc ccaggccc  
 1651 ctgctctctg ccgattgcca tcttcagcat gttggacaat cttcactgga  
 1701 ctctttgggg aaaaaaggcc ctctttccc tttccacccca atgaagctga  
 1751 ggagtgagaa taagaatctt cctgaaaattc taaaaaaaaa aaaaaaaaaa  
 1801 aaagagaacg ccttggccgt ggctgttcag ggcac  
 1851 gggacagcac agccgtggga tgaagcagcc tggggc  
 1901 gcagggtttt gcatgtctgg gtgaggtgtgg tgg  
 1951 ccaggccgtg gcgagggttag gggcacggct tct  
 2001 agccctggcc tcccttcaag gaggcttgc gatgc  
 2051 tttaaaaaaat tatctatttt atttattttt atttggtaa  
 2101 agggctcac tatgttgctc gggctggct caa  
 2151 tccctctgc ctcagcctcc gaaaggcttgc ggatt  
 2201 cactccggc ctgctcttagt ctttgc  
 2251 agaaaactt actccccacc aaccgc  
 2301 cccagactgg agtgc  
 2351 cccaggttca agcgatttcc  
 2401 cgggcacgc  
 2451 ttccaccatg ttggca  
 2501 ccacctcg  
 2551 cggctggat acagaaagct tttat

FIG.2A

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2601 caggcccattt ctggggttcc tcccaagtgg aattactgac ttaacattta  
 2651 gcttgggatc ctgagacttc catcacacag ttttctcatt gattgcagc  
 2701 caataatatac tgaaaaaaa acatctcagg ccgagcgctg tggctcacac  
 2751 ctgtaatcccc agcactttgg gaggctgagg tggcagatc acctgaggtc  
 2801 gggagtttga gaccagctg accaacaatgg agaaacccctg tctcttctaa  
 2851 aaaaatacaa aattagccag gcgtgggatc gcatgcctgt aatcccagca  
 2901 ctttgggagg ctgaggcagg agaatcgctt gaacccagga gacggaggtt  
 2951 ccgggtgagcc gagatcgccg cattgcactc cagccctggc aacaagagca  
 3001 aaactccgtc tcaaacaaac aaacaaaaaa catctctctg ctccttgggg  
 3051 ccgggtgcca gctctgctat tggaggact gaggcaccctt gaagcaggca  
 3101 tgtcactcct ctgtccccca gtttactcat ctgtaaatgg ggagagctgg  
 3151 ggcagacagt gagctggctg agggcaggac tggctctcctt caagccccatg  
 3201 gcccagggtt gccaggtatg agtttgtt cggtaaatgc tgctggcccc  
 3251 taagtgttag cggtccccctgc aaactgcagc gtatgggtgg acagccctgc  
 3301 acggctaccc ctttcttggg tgaccttatt tggttacggc cctatctgaa  
 3351 gtagggaaagg gacacttttg gctgtctctt agctccctca aggccccaca  
 3401 gcctggacta gagttgccag aaataacttgg tccattcagg ccaaaggac  
 3451 tgtgagggtt ctgggatggc gcaatcagtc ttgtccatg atgaacccac  
 3501 agggtagacc aggggttggg ccagcccaatgtt gcccctgtgtt gttgagccca  
 3551 gccccccaggc atcccatcccc gggcggtggc ctcaggtggc ggtggggcag  
 3601 ccagttgcca gggatgtt gcaatcggtca cctctcacca gccccggctg  
 3651 cccatcagct gtttcaatg ccaggcaatg aagcccttctt gccagggaaat  
 3701 tcccagagtt tctgtccat gaagtcaatc tggccatc ttggacaca  
 3751 agccgggtt ccctggggag agtactctgg gccccttggc aggtttgtct  
 3801 gagagtataa ggcagccctga tactagtggc gccagccagg gagggatgag  
 3851 gcccagccgc tgctggccat aagtataaa gggccatgtt ctgagtgcct  
 3901 actatgtgcc aggttttggaa atcagttactt gatttatgg aaccctctct  
 3951 ttaatccctc aagggtcccc tataggcacatcattt ttgttattgc  
 4001 cacttgacag atgaaaaaac agaggctcag agaggcaaaatggcttggaaa  
 4051 ttcaatggattt ggttgggat ttgaatccac agccatgtt ttaaggcat  
 4101 gctatgtgc cacatccatc gtttatttcc ggcactcattt gattcttcaa  
 4151 ttttgcactt attaaatcca tcagtgagca tcttctctgt gtcatgcatt  
 4201 gtttgcactt ctgaagatgt agctgtgagc aaaacttcta cagggatgaa  
 4251 gttcacagca gaggatcag cttagacaaa ggctcagagg tggaccgtg  
 4301 cgtccctgtt tccaggaata cagtatggct gcagcagaga gcagtggaga  
 4351 gaggccctgg cagtgaggc tagaggccgc cgggctggct catgctggat  
 4401 gtttgcactt tcggaaaggac tttggcttta tttaaagag gatggggagc  
 4451 cccagagagc acagcaggaa agcctgggaa gtctgatggc catttaaaag  
 4501 gatccttaat ggagagatgt aaggcagagc cttccagaag ggtaaagagaa  
 4551 gggaggatgg agacctgccc tcccccaagg gaggccactc agaagaggtt  
 4601 gagtggtggcc agggcagaga gcaagagagg ctgtggacac aggcacactg  
 4651 gtccatgtt agccatttgc cacatttgc ttagcttcat gtgtcttta  
 4701 gagagggagc cggctggcc tcgctctatg atcttggaca catcctttca  
 4751 cttctgggtc tcagtttccc cattatgtt atgaggatgtt gaatgtctt  
 4801 gtcctgggca cactatgggat gttggctggc gcacctgggt gcctgggttac  
 4851 catgggcaac aaagctctat tcatgggtt ggtgaatgca ttggccacag  
 4901 caactcaggc cggatgagga gtttcccagc agccctgggt gccccttccg  
 4951 ctgaaggccctt aacaactgtt ggaaaatcca agtcccagca gaccccccctga  
 5001 gcccctgtcc ttaggaccctt ctttcttagt gtttctctga gcctggccctg  
 5051 agctggagga gggagtgcc agtgcgtcag cagaggctgc ttcatagtaa  
 5101 ttgcagccaa cagttattgtt ctaggcactt ttctgggggg ttagatgtt  
 5151 gtaactgtt gaaatcgcc taaaactttt tgaggttaatgtt cctattgtt  
 5201 gcccatttttgc tagatgagga gactgagttt gaaactgggg ggtgtaatgg  
 5251 aacccatctca ggacccttgc agggtagggc ctttgcactc gggccacag

FIG.2B

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5301 ggtggggttt gtgtctgggt gggagctggg gagggacagg actaggatta  
 5351 ggcagatctg aggccacagg agttgggtgg ggggtggcgc cagagccact  
 5401 ccactccctc ctaccacatt gactgccttg aaagtcccct aatggccact  
 5451 cccatgaagt gtgactgctc tgggctcccc gcaggcggtt tctgcaaggc  
 5501 caccgcccac ccaggccccct tccccagagg ggctgcagtg cctgtctcc  
 5551 tccttgtggg aagagttggg ttgtctggc gtcagcaggta tactgcccct  
 5601 gggcatccct cccggtctct tcctgcgggt ttctgtatgaa acagccaggc  
 5651 tccagtagtg gagccagagg tcagtggtgg agagaggacc aggagccaga  
 5701 gggtatagct gcttggggc tactgtgggg tcagggacac ttgtgaggcc  
 5751 aagcgtctg gctgcaggag ccctcacata tatgcccacc cttcaccagg  
 5801 acattgaggg gtgctggggc acaggggttag ctttttgggg gtgtctgcct  
 5851 tcgacttggg ctccgcata caggccaaat ttggatgtcc catgtttaga  
 5901 gctgttttc tttgggacct ctggggcct cagttccctc atctgtaaaa  
 5951 tggatactg atagtgcctc cccactggcc tcctctgacg ggcgccagg  
 6001 agaggatggg acggagcatg gtgtgtggg cacgctctg ctgtacccac  
 6051 ccacctggga gaggggagag gcaggaatgt cctgggggtg tcctttgagg  
 6101 catagccctg tcaccccaac atcctacaaa ggcatgagaa ggcagcgagg  
 6151 acagaccccg accacctgag ccctcagcag ccctgcaca ctcctgtctt  
 6201 caccccttc ctgactgatc tggcacattc ttgattctcc tagggagtga  
 6251 cccaaaatcc ctccctgccc tgctgtgtct ctgggggtgga aggaggctgc  
 6301 cagccccctcc tctctcccaag cctcaggett ggccaggact taacaggcag  
 6351 gcagagaagc agcttctcca ctctcttccc tgacacctgt aggccccctcc  
 6401 tgcaggcact tacctctaag tggactctca ggaggaggct catcagggt  
 6451 gcagggctca gaaagagctg ggctgtggag ctcttgccaa ccgcccaggcc  
 6501 cttcttaagt gctttagcgc caccgactgc atcctcccaag cagccttgt  
 6551 agatggggat ttgtgtttcc cagttactg atgagaaata ctgtatgagag  
 6601 atgggtgtgg tcttgcgtgg ggctccctgg ctcctggata gcagctcagg  
 6651 ttcacattctg ggcaggctgg ctctggaca cccccccgc cagctgt  
 6701 gtgggattca cggtggggct tgggcagggc gtgggatctt gggccaaact  
 6751 gagccactt aggcttccag gaccaaggc caggctgagc tgctctgt  
 6801 tcctgagaga gcatgaacat cacagaagat gggcccggt tcaaatccca  
 6851 gctctgccac tactaactgg gacctggca ggggtccctt cccgctgagc  
 6901 cttcatttcc tcaccagcaa aatggttcg tggcctgttt tggggctgt  
 6951 ggagggttgg ctctgtcta tttgttcata cctgtgttg agcagctgt  
 7001 ctgtccggc ctctgaggat gccactgtga acagagctg tcgctaccc  
 7051 caggagctt gtttagggg tgccgtttt attccagcac tttcaccagg  
 7101 ctctgtctcc gtacccgatc agagacgtcg agtgcgcgtt tccactcg  
 7151 tgggtgcgtg tgggggttgg ggggacaggc ctttgcac gtagccctgg  
 7201 gtggatgttc ctgggtgcac ttaggggtgt tgagggtggg acctccaca  
 7251 gttcccttag gctccactga tgaggccaa gaaccgcctt cctgcccccc  
 7301 agcccaggt cccagcagct gggccctgg ctcttgaga tagtgaactgg  
 7351 cttcacggca aggacccccc cacaccaccc agggagaactg ctgctcccc  
 7401 tctgttccag gagtggcgac aagcacagtt tttcgctttt gttttgtt  
 7451 tcttcattt aagttccggg aaacgtgcag aatgtgcagg ttgttacat  
 7501 aggtatacat gtgccatggt gtttgcgtc acccgtaac ccctcatcta  
 7551 gttttaagc tccatataca ttaggcattt gtcctaattgc tctccctccc  
 7601 ctggccctc accggcccaag taagccccgg tgggtgtatgt tccctccct  
 7651 gtgtccatgt gttctcattt ttcaactctc acttatgagt gagaagagac  
 7701 ctggactctg atctaaccctc ggtcaaattgg aactgtgtga ctttgaaga  
 7751 gtagcttaac ctctgttagt cttagttct gcctggcacc cccatcctta  
 7801 aggagaggcc cacagaggac caggtcacat gacctcagcc agttccagag  
 7851 aaggctgttt gctccaggt ttccggcctga gtccaggccc ctgcccctact  
 7901 cgcactccct gatagcatga gaagcacagc cccagggtgc ccacccagct  
 7951 ctgagagccc agcctgcctt ccagggaaact gtcacagccc cacctgtccc

FIG.2C

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8001 ttccccagct ggagccctgt caatggcttt ggggttctct gacacagccc  
 8051 tgagggggct cacacttccc cttatcattg caaggggtag atctggcttg  
 8101 aaggccctgg ggcaggctt gttctgtctt cccctgtcag tgccctcgaca  
 8151 gggctggctt gggtaatca ggaccaacgg gaaaggaggc gaggagacca  
 8201 atctggaccc aagatcctca gctcaataag gtggcccccag aactgacatg  
 8251 gggtgataga gggaaaggctt gggagggagg agattctggg gccgcagcca  
 8301 cagcttgcac gttgcgcgg gtgtgtctgt gcgtgccagc tgcatcttg  
 8351 cgtaccatgt gtcaaggctt gtgtttggct gagtgttcat gtggccgtg  
 8401 attgtggcga tggcttgatg tggctgagtg atgcctgtct gtgtggctg  
 8451 gtgggtgtt ctgcattgtgc gtgtgtgtctt ggggagttt aaaggagaaa  
 8501 gagggactca ccattcacgtt ggctcagcct taaaaaaggta ggacatcctg  
 8551 acacgtgctt caacatggat ggacctaag gacattgtgc tgagtgaaac  
 8601 aagccagagg caaaggaaaca aacatgttat ttctcccaaga tgaggttcc  
 8651 ggaggaggca gatctgtatg gacagaaggtt agcatggtgg ttgcccggg  
 8701 agggggagga gagaatggag aattagtgtt taatggggac agagtttcag  
 8751 ttggggagga tggaaaggat ttggagctgg atgatggtga tggttggaca  
 8801 acactgtgca tgcacttaat accactgagc tggacaccta aaaatgctta  
 8851 caatggtaaa tttcatgtat attttactac aatttttaaa aaattggctg  
 8901 ggcgtggctt cttatgcctt taatcccaac actttggag gccaaggcgg  
 8951 gaggattgtt tgagctcagg agttcaacac cagcctggc aatatggtga  
 9001 aaccccgact ctacgaaata tacaaaaattt agcctgggtt ggtggcttgc  
 9051 acctctaattt ccacccatctc agtagctaa ggcacaagaa tctcttgaac  
 9101 ctgggagggtt gaggttgcag taagccgaga tcatgccact gcaacccagt  
 9151 ctgggcgaca gagcaagact ctgtctcaaa aaataaaaaga taaataaaaa  
 9201 aatttagagggc caggtgtggc tcacacctgt actctcaaca ctttggggagg  
 9251 ctgaggtggg aggatcgctt gaagtcaggc attaagaca tgccttaggc  
 9301 acatagttagt accttgactc tacaaaaaaa ttcaaaaattt aatggagacat  
 9351 ggtggcatgt gcctgttagtc cttagtgcgtt gggaggctga ggtggggagga  
 9401 tcacttacga ccaggattt aaggctgcag tgagctgtga ttgcataact  
 9451 gcactccagc ctggtgacag agtggggccc tgccttcaaaa aatttttca  
 9501 gtgtttttctt gggctggcg tgggtgctca ttccctgtat tccagcactt  
 9551 tgggaggctg aggtgggtgg attgttttag cccaggagtt taagaccagc  
 9601 tgggcaacat ggcaaacctt atcttacaa aaataaaaaaaa taaaaatatta  
 9651 gctgggcatg gtgggcaca cctgtactaa cagctacgag agaggctaaag  
 9701 gtgggaggat cacctgagcc cgggagggtt aggctgcagt gagccatgat  
 9751 tgcaccactg cactctagcc tgggcgatac agcaagaccc tatctcaaaa  
 9801 aaaaaaaaaaaa aaaaaaaaaaaa aaaaacaccc agtgggtca gttagaacc  
 9851 aagagtcttc ttccctccca gctccctgtt acaccagccc cagctctgca  
 9901 ggtagctggg ggcccagaca gcttcctggg gaccccccagc ttccctctg  
 9951 cccttttttcc taccagttt gctccccctc ttcaagact catgtccaga  
 10001 ggggggtgaga tctgcactta tacagcccccc tcctctgtaa tgagtgagcc  
 10051 aagtcaagccc agttattcc agaagggggca ccctaccagc cccccagtc  
 10101 ccaagctgcc ctgggcctat aaaagcaggc aaggggaccc ctagtagatc  
 10151 atgttaggtgt tacctcttag tgggtgctgg aggggcctga agtgccttct  
 10201 tccccccaggg tggtaggaga atgtcctggc agtgcattca gggcccgctg  
 10251 tcacttcgtt ttaagactc accagctggt aggctcatta gcaagaggac  
 10301 aataggagggc ccctgtccctc agtcaagctt cttcaaaggat gtttccttta  
 10351 gcaactggga ggccctccctt ctccagaccc atggggacaa caccacccag  
 10401 ctactgggtt tataagctgc tggatggctc tggctagccc attcagagaa  
 10451 agcctctgaa agtacaaggg aaaaaatcag tccaagagct gtgaacaatt  
 10501 agtgagccga ttacaatacc aagaccacag gcagacctgg aaggctaaatg  
 10551 gagcccaggt gtgaagttca agcttacttt acttctggc cacttcctgg  
 10601 ctggctcttt tccctggccc ttatcttctt cctggctctgt ttctctct  
 10651 caccctttt cttactctt tcttccttctt cctgcattcgt actccacccc

FIG.2D

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10701 cactccagct attacacaga atcgcgagaa tggtggatta ttcattttat  
 10751 ttatgatgtt ttctttttttaaaaaataga gacaaggctt cactatgtgg  
 10801 cccaggctgg tcttgaactc ctggcctcaa gcaatcctcg tgccttgccc  
 10851 tcttacagtg ctgggattac agatgtgagc caccatgcct ggcccattt  
 10901 atttactta aaaaaaaaaat taggttggc gcggtggctc acacctataa  
 10951 ttccagcaact ttgggaggcc aagggtggca gatcaactga ggtcaggagt  
 11001 taaagaccag ctggccacc tgggttgcagg agtttgcac cagctactcc  
 11051 ggaggcttag accggagaat tgcttgcacc caggaggtag aggttgcata  
 11101 gaactgagat catgccattt catggcagcc tggcaaacag agcaagactg  
 11151 tctcaaaaaa aaaaaaaaaat atgtttgtt ctcctgcttc ctgctttgtt  
 11201 agtcaaatca gttaactgt tcaagtgtt tccttgcaaa ccccccaagga  
 11251 ctcaatgtgt gtcgcctt actgatcccc ccggccctgt acccagttgtt  
 11301 cctcagttcc agttttccc acctaccctt caccactgc ttatgtttat  
 11351 aaaaacgggg taaatcaaattt gttcgtgacc cagatttat tctacatgca  
 11401 gtggaaactt gtatgactt agtttttgg aaaagcagaa ccttttttcg  
 11451 tggttcaaga aatcaaagtc ttcccgaggag gtcttctgt aaatccagag  
 11501 ctgcagatgt ttgaccgtgt tcagagaggg gccttgc tgggtgaagt  
 11551 ggatggggca cagcaggccaa tgggtgaaaaa gcaggacaac ctggggccct  
 11601 gggaggacca gggaggccccc atgttttgc ctgttcatca gcccggctgac  
 11651 ttccctgtccg cctgtcgctc gcttgcacc tccatccgta gtccttccgc  
 11701 ctgtctctgc tggttgcgc tggctactc agtgcgttgc gtcgttccgc  
 11751 ctgactgtct gcttccttc agGATGCCTT CCGTGCCTTC CATCAAGATC  
 11801 TCAATTGT GCGCAAGTTT CTACAGCCCC TGTTGATTGG AGAGCTGGCT  
 11851 CCGGAAGAAC CCAGCCAGGA TGGACCCCTG AATgtgagcc agagccctag  
 11901 gagaggctca gcccctgagg gagggggatg gctggaggc tgggagacat  
 11951 tgccacatgg ccaggagcag ctcctcgcc attcgcccaa ggggatgcag  
 12001 agccagggtt gaggctgccc tccctccca gggggcaggc agttgaaagt  
 12051 gaagctgttag ggatgcctt agaagtccag ggctccagat ctggtttagc  
 12101 caggcactcg tttggatccc gaggcaagct ccctccctgt tgcgcagg  
 12151 tgtcccccata aaaaggagga ttttgcattt ctgatttctc tcctggctgt  
 12201 agcgttttac ccacccata ccttttggg gggagaggag gcttcaccac  
 12251 cagccagtgc tccagctcac accccgggtt gggtactt gtcacttcat  
 12301 tcctcttgc ccacacccct tggcctggc gatgggagga gcccggccgg  
 12351 ctccaggaga atgggggtgg ggaggaattt cttcttggc tgatcgcccc  
 12401 ctctgcata gcaGCGCAG CTGGTCGAGG ACTTCCGAGC CCTGCACCAAG  
 12451 GCAGCCGAGG ACATGAAGCT GTTGTATGCC AGTCCCACCT TCTTGCTTT  
 12501 CCTACTGGGC CACATCCTGG CCATGGAGGT GCTGGCCTGG CTCCTTATCT  
 12551 ACCTCCTGGG TCCTGGCTGG GTGCCAGTG CCCTGGCCGC CTTCATCCTG  
 12601 GCCATCTCTC AGgtgacccc agttctgtgt tgccagccacc ttaactgccc  
 12651 aacagacgtg ggccccatg catctggca ttgtgaacat atttgcataa  
 12701 tgaatgaatg gacctatgaa aggatgaatg gatgaataaa cagatgaatg  
 12751 agtgaacagt ctgaaggccc atcaggcatg tctgtgggtc aagctgcatt  
 12801 ccagatgagc caagaaggcc cttcttgcac agatccgat caagcacagg  
 12851 gccactgagc cagaggctgc tggcctgcag cttcatgaca cttacgagcc  
 12901 cctccacctc cttggactc agttctcatc tgtaaaaaga ggacactggc  
 12951 ccacaagggtt ctgttttttgg agcatttagca cgggggttacc ctgcaagctg  
 13001 aaaggattca ctggggccccc aggcctggc gggctccgtc ttcccaaca  
 13051 gcttctgacc ctgcctctt ccccaGCTC AGTCCCTGGTG TCTGCAGCAT  
 13101 GACCTGGGCC ATGCCTCCAT CTCAGAAAG TCCTGGTGGA ACCACGTGGC  
 13151 CCAGAAGTTT GTGATGGGGC AGCTAAAGgt gagggtgggg tgggtggca  
 13201 gccagggtgtt ggggtggcgct gggctgcacc aagtgtgtgg gcacagtccg  
 13251 gggcacagcc tggccctgaga gccccctctt cttccacagG GCTTCCTCCGC

FIG.2E

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13301	<u>CCACTGGTGG AACTTCCGCC ACTTCCAGCA CCACGCCAAG CCCAACATCT</u>
13351	<u>TCCACAAAGA CCCAGACGTG ACGGTGGCGC CCGTCTTCCT CCTGGGGAG</u>
13401	<u>TCATCCGTCG AGgtgggtgg ggagggacct ggacaacctc tggctggcc</u>
13451	<u>tgcagctgag ggggagctaa tgcactgggt ccccaactcg cccctgac</u>
13501	<u>agccccctgat ctggcctcca ctctggctgg gccaagctct gccccctgt</u>
13551	<u>ctttccttcc cacctcccaa cctgctgggg acgaccagcc cgcttgctag</u>
13601	<u>aatctagagt tgccttgcac cttggcccc agccagcccc gtgacccctgc</u>
13651	<u>ccgggagaag gaggtggccc ggagagtcg tgcctccagc cgccgcctgt</u>
13701	<u>ctccacagTA TGGCAAGAAG AAACGCAGAT ACCTACCTA CAACCAGCAG</u>
13751	<u>CACCTGTACT TCTTCCTGAg tgagtgtcca tctgtccttc tgggtgggg</u>
13801	<u>gagtgcctgg gcctgcactg tcctccctgc tgcactggac cactccac</u>
13851	<u>cacttcctgg ggcggggcac gctgtcagg tctccctgg catggcatcc</u>
13901	<u>tcccagccctc tgcagtctgt acacacttc ccagcagcat gcctttgccc</u>
13951	<u>cagctgtctc ccgtgcctgg gacacccctgc agccacggc catcacagcc</u>
14001	<u>ctgctgggag ctttccaaag cccacatcg aatttcttct tgccctca</u>
14051	<u>agagtggtcc ggagccctag agtctttggg cagttgttgg ggcggacaga</u>
14101	<u>gtgaggactc aagtctggcc ctgacttgcg gtgaagggtg gtgggagggt</u>
14151	<u>gtgggttaag ggcagctgg ggaggcttgg acacagaatt ggggtgata</u>
14201	<u>tggggtcatt cagctggatg tgaccagcac caacgtccca ggggcattcc</u>
14251	<u>tggagtaaca gagccctca ctctggcgcc cactcacctt ggcagccag</u>
14301	<u>ccccactcct gaacactctc atgccccttc ttgcagTCGG CCCGCCGCTG</u>
14351	<u>CTCACCCCTGG TGAACTTGA AGTGGAAAAT CTGGCGTACA TGCTGGTGTG</u>
14401	<u>CATGCAGTGG GCGgtgagtg gggttgc cca ggaccccgaa catacggtcg</u>
14451	<u>ccgtggcagg aggtgggtgcc tcgggggaca gtacccgtccc atgaaggca</u>
14501	<u>acagggtgca catgtgcgtg caacagtgtg gtcacatgt atgcgtgaa</u>
14551	<u>cagtgtggct cacatgtgtg cgcgcagcag gagagcgagt gtcccgta</u>
14601	<u>ctgtacgtgt ggtgggggg ggtttagggaa caggggggt gtgggtctct</u>
14651	<u>ctcggtgagg gtgtcttccc aggaggagtt gctggccga ctctgccagg</u>
14701	<u>catctgtgtc cctggcaggg tcttcccaa cacaccctgc atgacaccc</u>
14751	<u>cgtcactaaa atcagccctcg tgagctggca gggcaaggac cctgttcctt</u>
14801	<u>tactcagctg agaaaaccag agagggttgt ggcctgtct gggctctgag</u>
14851	<u>gcaaatcagg cagaagggtt ggatgcctga ggtcctctc ccaccacca</u>
14901	<u>ggcctccaga cctccggca cctggagacc tctcggtatc gcctctgccc</u>
14951	<u>tcctctgcag GATTGCTCT GGGCCGCCAG CTTCTATGCC CGCTTCTTCT</u>
15001	<u>TATCCTACCT CCCCTCTAC GGCGTCCCTG GGGTGTGCT CTTCTTGTG</u>
15051	<u>GCTGTCAGgt atggcaggga gtggcgaggt cacacacagg cgacaggtga</u>
15101	<u>cccccaactgc agccccccac caagacttcc ctttccctgt ctgcagaatg</u>
15151	<u>gggcctagtgg tactgcctcc ctggcttgc ggtgaatca cataaacaca</u>
15201	<u>agcgtggcag gagcccaagg tcgggtgggt tagggagcgt ggcctggctt</u>
15251	<u>gtaagtggcc cgggtgggtgt cggagctgt ctggactcag ctcacacgt</u>
15301	<u>gacactgctc cattcagatt cttaaacac tggcaagggg gcgatggca</u>
15351	<u>caatcctatt gtacagataa ggaagtcaag gccacttggg gacagctgct</u>
15401	<u>ctccagccctc cactcagggt gcctaagtgg tgagctggac ctaggcagt</u>
15451	<u>gcccggccct ccccacagGG TCCTGGAAAG CCAACTGGTT GTGTGGATCA</u>
15501	<u>CACAGATGAA CCACATCCCC AAGGAGATCG GCCACGAGAA GCACCGGGAC</u>
15551	<u>TGGGTCAAGCT CTCAGgtggg cagcagggtt gggcccatc ctgggtgggg</u>
15601	<u>tgggggtcc cagctaggag ccagatggca aagcaggat gaggccctga</u>
15651	<u>cggggctgccc aggtggggga tggccctgg ggtcaggga tctgcaacgg</u>
15701	<u>cctccctaca tggcccccgc cggcttccgg cagCTGGCAG CCACCTGCAA</u>
15751	<u>CGTGGAGCCC TCACCTTCA CCAACTGGTT CAGCGGGCAC CTCAACTTCC</u>
15801	<u>AGATCGAGCA CCAgtgagtg tgggtgtgg gggccagtgg gaggtgggg</u>
15851	<u>gggggtcctg ggagggatc ctgggggggg acccgtgggt gggcctctc</u>

FIG.2F

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15901 tctggaatct cccacttcag gtgccagcat acgctccccca ccccccagCCT  
 15951 CTTCCCCAGG ATGCCGAGAC ACAACTACAG CCGGGTGGCC CGCGCTGGTCA  
 16001 AGTCGCTGTG TGCCAAGCAC GGCCCTCAGCT ACGAAGTGAA GCCCTTCCTC  
 16051 ACCGCGCTGG TGGACATCGT CAGgtgaggc tgcagccggg cccctctgtt  
 16101 ctgggtggctt ccccagggcc tatgcctacc cttgtccagg tcagcctat  
 16151 gctgagcccc cagggtccct gagcctttct gtccacgtcc catgcccttc  
 16201 ctcccttccc cagccttcac gcacacagtg agaattctcg gagcacctac  
 16251 tgcagactca caaacagcag tgccctgggt gaggcgtct atgcaaaacct  
 16301 accccccaaag gctgaggggaa aaaagctaac agatccagtt tctcagaagg  
 16351 aaacacttaa caggactca taaacagaag ccatgtctca gggccgggtg  
 16401 cggtggtctca cgccgtataat tccagcactt ggggaggctg aggtggcg  
 16451 atcaactttag gtcaggaggt cgagaccagg ctggccaaca tggtaaaacc  
 16501 cccgtctctac taaaaaaaaaaa aaaaaaaaaac aaaacaaaac aaaaattagc  
 16551 tgggtgtggt ggcaggtgcc cataatccca gctacttggg aggctgaggg  
 16601 aggagaatca cttgaactcg caggggcaga gggtgcagtg agctgagatt  
 16651 gtgccttgc agtccagcct gggcaacacaga gcaagactct ctcaaaaaaca  
 16701 aaaaaaaaaa ccatgtctca ggcagccaag agttgggaca tcccctcaca  
 16751 cggccctctag aaagaaccct ctatatacgca agcttttagg gtgaacccca  
 16801 tgcaggttgt tcttatgaac ctgggtacca ctggaggtt gataagcgtc  
 16851 tacaagagga gtttatctat gccatgagct tggcattcag ggtcaagcat  
 16901 cggtcatctag acagtttgc ttgaagatgg cattgccctt gtagcaatgc  
 16951 aggctctaga gagttccctg ccctcttggg gctgatgttc ctccagcaa  
 17001 agaaaaacgc aagcaattaa aataacaaat aagtacatta cagaagatgg  
 17051 gcaaaaagaac aatgaaaagc ccctcagggg ggggacaggg gaggggaggg  
 17101 gggcgcccag gcagggggcgg cagttctaa ataggtggta ggggtggcag  
 17151 tattgacagg ctgacgtgtg agcagggaca gggaggaggg gagaggtctc  
 17201 gccacagggg catctggcaa agagcgttca ggcagagggc acttgaccct  
 17251 gaatgccaag ctcatggcat agatagccga ggcaggcatg caggcactca  
 17301 gagaagggac acgcccggct tgcattttgg aaagctgccc ctactggaa  
 17351 tgactggcgg gcaggagtcg aagtggaaaa ggagagcaga ggacactgca  
 17401 gccatccagg cgaggggtga tggggctcag cccttgggt caccttgg  
 17451 gtggggaaaca gaggccagat tccaggtctt ataccttgc ggtgttcaga  
 17501 acgctgttcc cttacttgg ttgcccttc ttccctgtct tgagccctg  
 17551 tgccccacttc tccttcatga tctctcccaag cctgatgttc  
 17601 ccattttggca cagccctta gagcgcctgg cacaggctt  
 17651 tggtgacatt tctggctcca ctgccccata tcagccccaa gatcggttgg  
 17701 gcaggttcca cgtcctcttgc tgccttgggt tgcaaggccc agcaggaggc  
 17751 agcaatggag aactgggtgc aggagggaca gggccaccca ggctcatgcc  
 17801 tggacttggc cttggctgcc ctccagctcc cttacccgac acccgtcacc  
 17851 ccggctctaga ttccatttcca gagaatgagc attcagctgt tctcccaacc  
 17901 caccctccag cccgcattcgc tgcctggccc caggaaaggg aacccacagg  
 17951 gaatggggat ctccgctcac atttaccatg ggggatacag ggggtttagg  
 18001 atcttgcacac tgagctctta acacccaccc ccactgcccac ccccacctcc  
 18051 cagGTCCCTG AAGAAGTCTG GTGACATCTG GCTGGACGCC TACCTCCATC  
 18101 AGTGAAGGCA ACACCCAGGC GGGCAGAGAA GGGCTCAGGG CACCAGCAAC  
 18151 CAAGCCAGCC CCCGGCGGGGA TCGATACCCC CACCCCTCCA CTGGCCAGCC  
 18201 TGGGGGTGCC CTGCCTGCC TCCTGGTACT GTTGTCTTCC CCTCGGCC  
 18251 CTCACATGTG TATTCAAGCAG CCCTATGGCC TTGGCTCTGG GCCTGATGGG  
 18301 ACAGGGGTAG AGGGAAGGTG AGCATAGCAC ATTTCCTAG AGCGAGAATT  
 18351 GGGGAAAAGC TGTTATTTT ATATTAAT ACATTCAGAT GTATTATGGA  
 18401 GT

FIG.2G

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1	CTTCGCTTCCCTCGGGGTCTTGCTCGGACCTCGGCCACCGCCTGGGATCC	50
51	CCAGGACTCGTGCCTGCAGCATGGCGGCGTCGGGAGCCGGACCGCGG 1 M G G V G E P G P R	100 10
101	GAGGGACCCGGCGCAGCCGGGGCACCGCTGCCACCTTCTGCTGGGAGCA 11 E G P A Q P G A P L P T F C W E Q	150 27
151	GATCCGCGCGCACGACCAGCCCAGCGACAAGTGGCTGGTCATCGAGCGCC 28 I R A H D Q P G D K W L V I E R R	200 44
201	GCGTCTACGACATCAGCCGCTGGGCACAGCGGCACCCAGGGGCAGCCGC 45 V Y D I S R W A Q R H P G G S R	250 60
251	CTCATCGGCCACCACGGCGCTGAGGACGCCACGGATGCCCTCGTGCCTT 61 L I G H H G A E D A T D A F R A F	300 77
301	CCATCAAGATCTCAATTGTGCGCAAGTTCCTACAGCCCTGTTGATTG 78 H Q D L N F V R K F L Q P L L I G	350 94
351	GAGAGCTGGCTCGGAAGAACCCAGCCAGGATGGACCCCTGAATGCGCAG 95 E L A P E E P S Q D G P L N A Q	400 110
401	CTGGTCGAGGACTTCCGAGCCCTGCACCAGCAGCCGAGGACATGAAGCT 111 L V E D F R A L H Q A A E D M K L	450 127
451	GTTTGATGCCAGTCCCACCTTCTTGCTTCTACTGGGCCACATCCTGG 128 F D A S P T F F A F L L G H I L A	500 144
501	CCATGGAGGTGCTGGCCTGGCTCCCTATCTACCTCCTGGTCCTGGCTGG 145 M E V L A W L L I Y L L G P G W	550 160
551	GTGCCCAAGTGCCCTGGCCGCTTCATCCTGGCCATCTCTCAGGCTCAGTC 161 V P S A L A A F I L A I S Q A Q S	600 177
601	CTGGTGTCTGCAGCATGACCTGGGCCATGCCCATCTCAAGAAGTCCT 178 W C L Q H D L G H A S I F K K S W	650 194
651	GGTGGAAACCACGTGGCCCAGAAGTTCGTGTGGGCAGCTAAAGGGCTTC 195 W N H V A Q K F V M G Q L K G F	700 210

FIG.3A

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701	TCCGCCCACTGGTGGAACTTCCGCCACTTCCAGCACCACGCCAAGCCCAA	750
211	S A H W W N F R H F Q H H A K P N	227
751	CATCTTCCACAAAGACCCAGACGTGACGGTGGCGCCCGTCTTCCTCTGG	800
228	I F H K D P D V T V A P V F L L G	244
801	GGGAGTCATCCGTCGAGTATGGCAAGAAGAACGCAGATACTACCCCTAC	850
245	E S S V E Y G K K R R Y L P Y	260
851	AACCAGCAGCACCTGTACTTCTTCCTGATCGGCCCGCCGCTGCTCACCT	900
261	N Q Q H L Y F F L I G P P L L T L	277
901	GGTGAACCTTGAAAGTGGAAAATCTGGCGTACATGCTGGTGTGCATGCAGT	950
278	V N F E V E N L A Y M L V C M Q W	294
951	GGGCGGATTGCTCTGGGCCGCCAGCTCTATGCCCGCTCTCTTATCC	1000
295	A D L L W A A S F Y A R F F L S	310
1001	TACCTCCCCCTCTACGGCGTCCCTGGGTGCTGCTCTCTTGTTGCTGT	1050
311	Y L P F Y G V P G V L L F F V A V	327
1051	CAGGGTCCTGGAAAGCCACTGGTCGTGGATCACACAGATGAACCACA	1100
328	R V L E S H W F V W I T Q M N H I	344
1101	TCCCCAAGGAGATGGCCACGAGAACGGACTGGGTAGCTCTCAAG	1150
345	P K E I G H E K H R D W V S S Q	360
1151	CTGGCAGCCACCTGCAACGTGGAGCCCTCACTTTCACCAACTGGTCAG	1200
361	L A A T C N V E P S L F T N W F S	377
1201	CGGGCACCTCAACTTCCAGATCGAGCACCACCTCTCCCCAGGATGCCGA	1250
378	G H L N F Q I E H H L F P R M P R	394
1251	GACACAACTACAGCCGGGTGGCCCGCTGGTCAAGTCGCTGTGTGCCAAG	1300
395	H N Y S R V A P L V K S L C A K	410
1301	CACGGCCTCAGCTACGAAGTGAAGCCCTCCTCACCGCGCTGGTGGACAT	1350
411	H G L S Y E V K P F L T A L V D I	427
1351	CGTCAGGTCCCTGAAGAAGTCTGGTGACATCTGGCTGGACGCCCTACCTCC	1400
428	V R S L K K S G D I W L D A Y L H	444

FIG.3B

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1401	ATCAGTGAAGGCAACACCCAGGCAGGGCAGAGAAGGGCTCAGGGCACCA	1450
445	Q	445
1451	AACCAAGCCAGCCCCGGCGGGATCGATA	1500
1501	CCCCCACCCTCCACTGGCCA	1550
1551	GCCTGGGGGTGCACTGCCTGCCCTCCCTGGTACTGTTGTCTTCCCTCGGC	1600
1601	CCCCTCACATGTGTATTCA	1650
1651	GAGCAGGGTAGAGGGAAGGTGAGCATA	1700
	GCACATTTCTAGAGCGAGA	
	ATTGGGGAAAGCTGTTATTTTATATTA	
	AAAATACATTCA	
	GATGTAAAAA	

FIG.3C

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1	GTACAGCGGCAATGGCGGTGTCGGGAGCCGGAGGGGACTCGGGCG	50
1	M G G V G E P G G G L G P	13
51	CGGGAGGGGCCGCACCGCTGGGGCGCCCTACCCATCTCCGCTGGGA	100
14	R E G P A P L G A P L P I F R W E	30
101	GCAGATCCGCCAGCATGACCTACCAGGCACAAGTGGCTGGTCATCGAGC	150
31	Q I R Q H D L P G D K W L V I E R	47
151	GCCGTGTCTACGACATCAGCCGCTGGGACAGCGGCACCCAGGGGTAGC	200
48	R V Y D I S R W A Q R H P G G S	63
201	CGCATCATGGCCACCA CGG	220
64	R I I G H H	69

FIG.4

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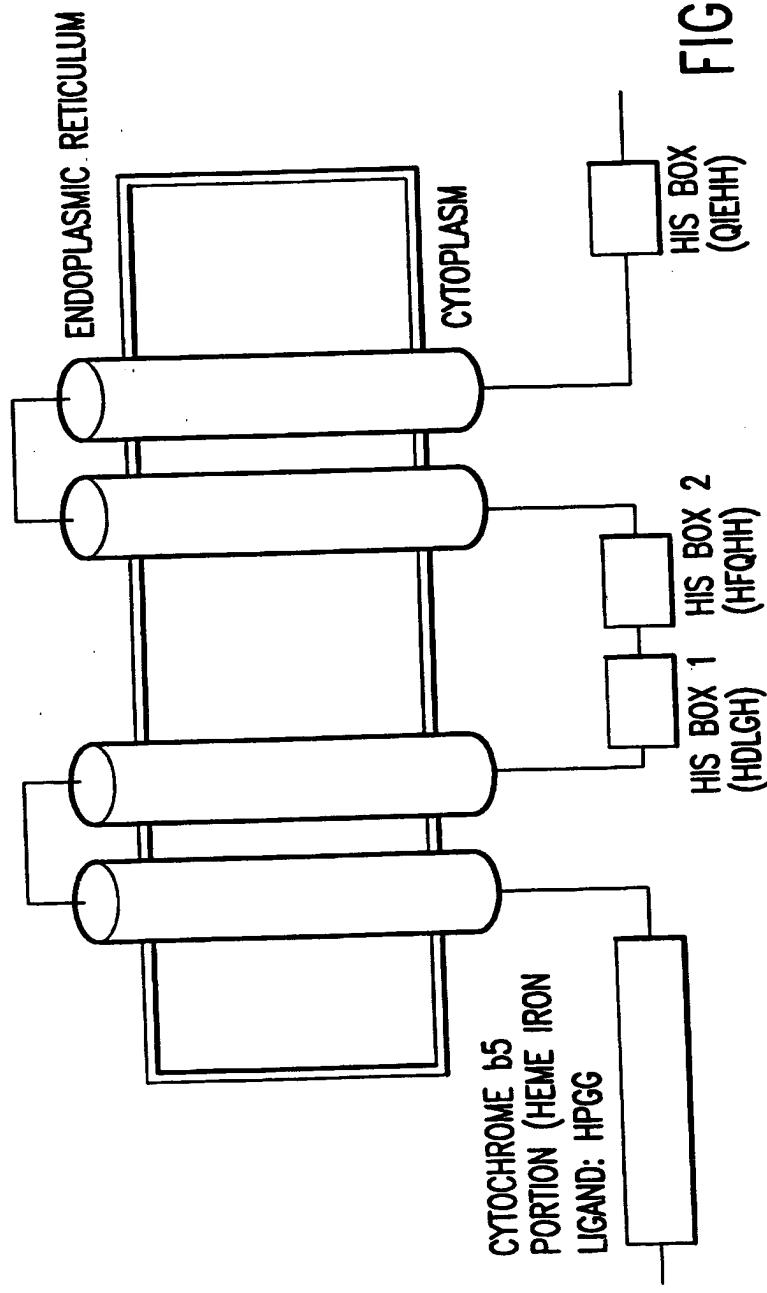
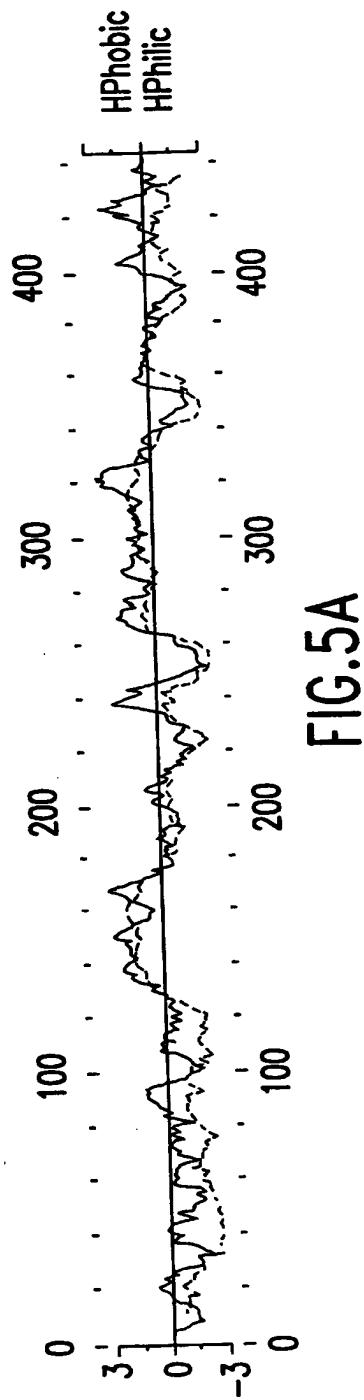


FIG. 5B

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PROFILESCAN of : CYB5rp\_correct\_protein check: 5714 from: 1 to: 445

GETSEQ from bmd, December 2, 1997 14:20.

Compare to profile library: GenRunData:profilescan.fil

---

Profile: profiledir:cytochrome\_b5.prf

Gap weight: 4.50 Gap Length weight: 0.05

Ave match: 0.27 Ave mismatch : -0.21

(Peptide) PROFILEMAKE v4.40 of: 0191.Msf2{} Length: 48

Sequences: 24 MaxScore: 27.58 December 2, 1992 00:07

This profile is derived from PROSITE release 10.0 and has been tested by a database search against SWISS-PROT release 26.0. A comparison of the SWISS-PROT annotation and the results of the database search follows. For further information about this motif, consult the . . .

Profile: profiledir:cytochrome\_b5.prf alignment: 1

Quality: 20.77 Gaps: 0

Ratio: 0.43 Length: 48

Normalized quality: 2.91

S 31 HDQPGDKWLVIERRVYDISRWAQRHPGGSRLIGHHGAEDATDAFRFH 78

|: ...: ||||. .|||::| . ||||. | .||.|:||. | ::|

P 1 HNDGEETWLVNGQVYDITKFLEEHPCGPDVIMEAAGTDATEEFEAIH 48

\*\*\*\*\*

\*Cytochrome b5 family, heme-binding domain signature \*

\*\*\*\*\*

FIG.6

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pir:s68358 hypothetical protein - common sunflower  
 Length = 458

Score = 169 (79.4 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42  
 Identities = 31/85 (36%), Positives = 49/85 (57%) His box 3

Query: 348 IGHEKHRDWVSSQLAATCNVEPSLFTNWFSGHLNFQIEHHLFPRMPRHNYSRVAPLVKSL 407  
 +G K +W Q T ++ S + +WF G L FQ+EHHLFPR+PR + ++P+ + L  
 Sbjct: 348 VGPPKGDNWFEKQTRGTIDIACSSVMDWFFCGGLQFQLEHHLFPRLPRLPRCHLSISPICREL 407

Query: 408 CAKHGLSYEVKPFLTALVDIVRSLK 432  
 C K+ L Y F A V ++L+  
 Sbjct: 408 CKKYNLPYVSLSYDANVTLKTLR 432

Score = 133 (62.5 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42  
 Identities = 21/53 (39%), Positives = 35/53 (66%)

HPGG motif

Query: 26 EQIRAHQPGDKWLVIERRVYDISRWAQRHPGGSRLIGHHGAEDATDAFRASH 78  
 +++++ H+ P D W+ I +VY+++ WA+ HPGG + + +D TDAF AFH  
 Sbjct: 22 KELKKHNNPNDLWISILGKVYNVTEWAKEHPGGDAPLINLAGQDVTDFAFIHF 74

Score = 118 (55.5 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42  
 Identities = 25/76 (32%), Positives = 34/76 (44%)

His box 1His box 2

Query: 165 LAAFILAISQAQSCLQHDLGHASIFKKSWWNHVAQKFVMGQLKGFSAHWWNFRHFQHEA 224  
 L+ IL ++ Q L HD GH + WN A F+ + G S WW + H HH  
 Sbjct: 152 LSGAILGLAWMQIAYLGHDAHYQMMATRGWNKFAGIFIGNCITGISIAWWKWTNAHII 211

Query: 225 KPNIFHKDPDVTVAPV 240  
 N DPD+ P+  
 Sbjct: 212 ACNSLDYDPDLQHLP 227

Score = 34 (16.0 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42  
 Identities = 7/14 (50%), Positives = 9/14 (64%)

FIG. 7A

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gp:bou79010\_1 PID:g2062403 *Borago officinalis* delta 6 desaturase mRNA,  
complete cds. (gb:U79010) (NID:2062402)  
Length = 448

Score = 179 (84.1 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42

Identities = 34/87 (39%), Positives = 48/87 (55%)

### His box 3

Query: 348 IGHEKHRDWSSQLAATCNVEPSLFTNWFSCHLNFQIEHHLFPRMPRHNSRVAPLVKSL 407  
+G K +W Q T ++ + +WF G L FQIEHHLFPRMPRHNSRVAPLVKSL

Sbjct: 338 VGKPKGNWWFEKQTDGTLDISCPPMDWFHGGLQFQIEHHLFPKMPPRCNLRKISPYVIEL 397

Query: 408 CAKHGLSYEVKPFLTALVDIVRSLKKS 434  
C KH L Y F A +R+L+ +

Sbjct: 398 CKKHNLPYNYASFSKANEMTLRTLRTN 424

Score = 144 (67.7 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42

Identities = 23/53 (43%), Positives = 36/53 (67%)

### HPGG MOTIF

Query: 26 EQIRAHDPQPGDKWLVIERRVYDISRWAQRHPGGSRLIGHGAEDATDAFRAFH 78  
+++ HD+PGD W+ I+ + YD+S W + HPGGS + ++ TDAF AFH

Sbjct: 12 DELKNHDKPCDLWISIQQKAYDVSDDWVKDHPGGSFPLKSLAGQEVTDAFVAFH 64

Score = 105 (49.3 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42

Identities = 22/68 (32%), Positives = 28/68 (41%)

### His box 1

### His box 2

Query: 176 QSWCLQHDLGHASIFKKSMWNHVAQKFVMGQLKGFSAHWNFRHFQHHAKPNIFHKDPDV 235  
QS + HD GH + S N F L G S WW + H HH N DPD+

Sbjct: 153 QSGWICHDAWHYVVSDSRLNKFMGIFAANCLSGISIGWWKWNNAHHIACNSLEYDPDL 212

Query: 236 TVAPVFL 243

p ++

Sbjct: 213 QVIPFLWV 220

FIG. 7B

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pir:s35157 Delta(6)-desaturase - Synechocystis sp.  
Length = 359

Score = 126 (59.2 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09

Identities = 21/54 (38%), Positives = 33/54 (61%)

His box 3

Query: 372 FTNWFSGHLNFQIEHHLFPRMPRHNSRVAPLVKSLCAKHGLSYEVKPFLTALV 425  
F NMF G LN Q+ HLF P + . +Y ++ ++K +C + G+ Y+V P A +

Sbjct: 292 FWNWFCCGLNHQVTHLFPNICHIHYPQLENIKDVCQFGVEYKVYPTFKAAI 345

Score = 36 (16.9 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09

Identities = 6/15 (40%), Positives = 8/15 (53%)

His box 2

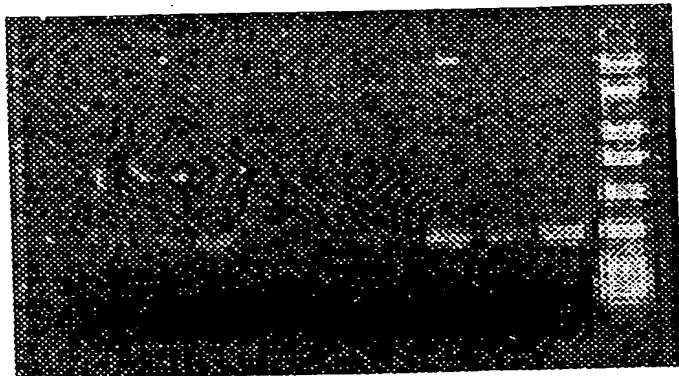
Query: 209 GFSAHWWNFRHFQHH 223  
G S+ W +RH H

Sbjct: 113 GLSSFLWRYRHNYLH 127

FIG.8

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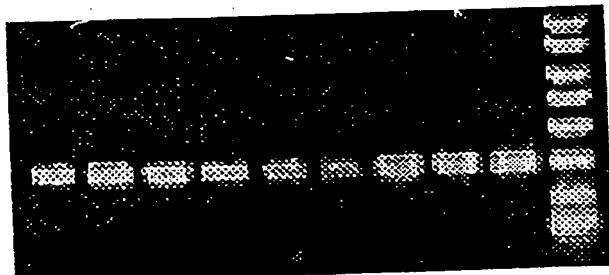
1 2 3 4 5 6 7 8 9



- |             |                    |
|-------------|--------------------|
| 1. Heart    | 6. Skeletal Muscle |
| 2. Brain    | 7. Kidney          |
| 3. Placenta | 8. Pancreas        |
| 4. Lung     | 9. Retina          |
| 5. Liver    |                    |

**FIG.9A**

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1 2 3 4 5 6 7 8 9 L PCR Marker

- |             |                    |
|-------------|--------------------|
| 1. Heart    | 6. Skeletal Muscle |
| 2. Brain    | 7. Kidney          |
| 3. Placenta | 8. Pancreas        |
| 4. Lung     | 9. Retina          |
| 5. Liver    |                    |

FIG.9B

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/23253

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 9/395; C12P 7/62; C12N 9/02, 15/00; C07H 19/00  
US CL :435/135, 189, 320.1, 452.3; 424/130.1; 536/23.2

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/135, 189, 320.1, 452.3; 424/130.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline

Search terms: CYB5RP, delta<sub>5</sub> fatty acid desaturase, human or homo sapiens.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank, Accession AAC23396, submitted by LAMERDIN, JE, publicly available on 12 June 1998, see entire record.	1-15
X	Database GenBank, Accession AC004770, submitted by LAMERDIN, JE, publicly available on 12 June 1998, see entire record, especially identification of CDS at about line 50.	1-15
X,P	Database GenBank, Accession AAD31282 submitted by LI et al, publicly available on 19 May 1999, see entire record.	1-15
X	WO 98/39446 A2 (HUMAN GENOME SCIENCES, INC.) 11 September 1998, see entire document, especially SEQ ID No:63.	1-15

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Z"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 FEBRUARY 2000

Date of mailing of the international search report

15 MAR 2000

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**INTERNATIONAL SEARCH REPORT**

International application No. PCT/US99/23253
---

**B. FIELDS SEARCHED**

Documentation other than minimum documentation that are included in the fields searched:

Because a CRF was not made available at the time of the search, Database GenBank Accession AF134404, which appears to encode the same desaturase as set forth in Figures 3A-C of the instant application, was searched against all available amino acid and nucleic acid databases.

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